Therapy – Intraarticular

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HEPARIN-BINDING IGF-1 PROVIDES SUSTAINED DELIVERY OF IGF-1 TO CARTILAGE IN VIVO

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Purpose: IGF-1 stimulates cartilage growth and repair but is not a practical therapy due to its short half-life in vivo. We modified IGF-1 by adding a heparin-binding domain, creating HB-IGF-1. HB-IGF-1 is retained in bovine cartilage tissue and promotes sustained proteoglycan synthesis compared to IGF-1.
In the present study, we examined the mechanism of HB-IGF-1 retention in cartilage and tested whether HB-IGF-1 could provide sustained in vivo delivery of IGF-1 to rat cartilage after intra-articular injection.
Methods: *Protein Production:* Both HB-IGF-1 and control IGF-1 were expressed with Xpress and 6x-His tags in E. coli and purified by Ni-NTA affinity and reverse-phase chromatography.
Bovine Cartilage with Enzyme Treatment: Cartilage disks (3 mm diam, 0.5 mm thick) from calf femoropatellar grooves were cultured in serum-free medium with 500nM HB-IGF-1 or IGF-1 for 2 days. At Day 2, disks were washed and treated with either no enzyme, chondroitinase ABC (0.4U/mL), or heparitinase (0.036U/mL). At Day 4, half of the chondroitinase-treated disks were treated with heparitinase; all other disks were incubated in enzyme-free medium. On Day 6, IGF remaining in the tissue was detected by Western analysis.
CHO cell binding: Mutant CHO cells lacking heparan sulfate (strain pgsD-677) and wildtype CHO (K1) cells were incubated in serum-free medium with 100nM HB-IGF-1 or IGF-1 for 3 h, washed, and analyzed by Western.
Biacore: Chondroitin sulfate (CS) and heparan sulfate (HS) were biotinylated and bound to a streptavidin-coated Biacore chip. HB-IGF-1 or IGF-1 was flowed across the chip to determine the maximum amount of IGF-1 bound (RUmax).
Intra-articular injection in rat: 10ug HB-IGF-1, 10ug IGF-1, or saline alone was injected into the knee joints of 2-month-old male Sprague-Dawley rats. After one day, joint tissues were harvested and extracted. Portions of extracts with equal total protein were analyzed by Western.
Results: HB-IGF-1 but not IGF-1 remained bound to bovine cartilage explants after 6 days (Fig. 1, No Enzyme). Treatment with chondroitinase ABC greatly decreased binding, while heparitinase had no effect (Fig. 1, C'ase and H'ase). In addition, HB-IGF-1 bound CHO cells lacking HS equally well as wild-type cells, indicating that HS is not required for binding. Biacore analysis showed that HB-IGF-1 bound to both HS and CS at 250-500nM, although HS binding was stronger than CS binding (Fig. 2). Control IGF-1 did not bind any of the GAGs at concentrations up to 1 uM. One day after intra-articular injection, HB-IGF-1 was retained in rat knee articular cartilage, whereas IGF-1 was undetectable (Fig. 3, Articular Cartilage). HB-IGF-1 was detectable despite stronger immunoreactivity of IGF-1 (Fig. 3, Protein Standards). Neither IGF-1 was detected in patellar tendon extracts (Fig. 3, Tendon), consistent with better delivery to the CS-rich cartilage.

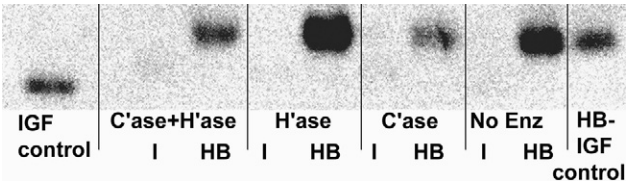

Figure 1

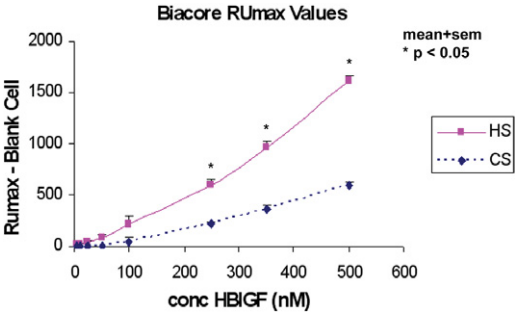

Figure 2

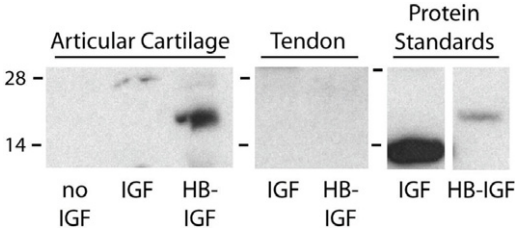

Figure 3

Conclusions: HB-IGF-1 is retained in rat knee cartilage longer than IGF-1 after intra-articular injection. Surprisingly, although HB-IGF-1 binds most strongly to heparan sulfate, the enhanced retention of HB-IGF-1 in cells and cartilage appears primarily due to binding of chondroitin sulfate, which is much more abundant in cartilage than HS. HB-IGF-1 may be a new therapeutic for sustained and relatively specific delivery of IGF-1 to cartilage.

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A SINGLE INTRA-ARTICULAR INJECTION OF INTERLEUKIN 1 RECEPTOR ANTAGONIST IS INSUFFICIENT TO RESTORE GAIT DEFICIENCY RESULTING FROM INTERLEUKIN-1 MEDIATED CARTILAGE DESTRUCTION IN A RODENT MODEL

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Purpose: Interleukin-1 (IL1) plays a pivotal role in osteoarthritis (OA), regulating inflammatory and catabolic processes. IL1 receptor antagonist (IL1Ra) can block IL1-induced inflammation in rheumatoid arthritis, and intra-articular injection of IL1Ra has been evaluated as an OA treatment. IL1-mediated joint inflammation and destruction has been modeled in the rat through intra-articular injection of rat dermal fibroblasts modified to over-express human IL1β. Here, we examine the efficacy of a single intra-articular IL1Ra injection in reversing pain and functional loss in this model.
Methods: Pre-treatment measures of mechanical sensitivity and gait were determined for male Wistar rats (n=14, day=-2). Right knee joints then received a 30 μL injection containing 12,500 rat dermal fibroblasts modified to overexpress human IL1β (day=-1). The following day, rats received either a 30 μL intra-articular injection of IL1Ra (0.65 mg/mL) or saline (n=7, day=0). Gait was quantified on post-treatment day 2 and 6 using high-speed video, and mechanical withdrawal thresholds were measured on post-treatment day 1, 3, and 5.
Results: Following injection of IL1β overexpressing cells, rats locomoted with faster velocities and longer stride lengths on post-treatment day 2 and 6 relative to pre-treatment data ($p<0.001$); however, rats treated with IL1Ra selected slower velocities than rats receiving saline ($p=0.056$). Rats receiving saline demonstrated asymmetric gait on post-treatment day 2 and 6 with a delayed time